indirectly to slow embryonic development by preventing lutein cells from functioning maximally. A similar mechanism has been postulated as the cause of embryonic diapause in the tammar wallaby *Macropus eugenii* (*7*). Alternatively, diapause may be caused by the temporary lack of some factor in the uterine environment, such as a uterine protein that governs blastocyst growth (*8*).

Although not a common reproductive pattern in bats, delayed implantation or development is known to occur in several other species of Chiroptera. Only one nonhibernating species, *Eidolon helvum* (family Pteropidae) from Uganda (0°), is known to undergo delayed implantation (*9*). Females of this frugivorous species are fertilized in April or May, but implantation does not occur until October or November; a single young is born in February or March. The time of both implantation and birth coincide with peaks in rainfall. In the "quasi-hibernating," temperate-dwelling phyllostomid *Macrotus waterhousii*, fertilization occurs between September and November, and embryonic development proceeds slowly during the winter; the young are born in June (*10*). Wimsatt (*11*) inferred from Bradshaw's incomplete account that this may represent another case of delayed implantation, but definite proof of this is lacking. As a final example, *Miniopterus schreibersii* (family Vespertilionidae), a true hibernator, displays delayed implantation during its period of winter dormancy (*11*). In this species, fertilization occurs before the bats enter hibernation, but implantation does not occur until after spring arousal so that the young are born in the early summer.

Although the ecological settings of the different bats which display delayed implantation or development are varied, the timing of events is such as to allow young to be born at energetically favorable times of the year. This seems to be the case in *A. jamaicensis*. Blastocysts conceived in Panama in July through September and developing directly would result in births occurring in November through January. This means that females would be pregnant or lactating and young would be weaned at times when the availability of fruit is relatively low. Therefore, delayed development allows the young to be born, and the females to be most active at a time when fruit is most plentiful (*12*) (Fig. 3). Although the adaptive value of delayed development in *A. jamaicensis* seems relatively clear, the proximate factors behind this adaptation are unknown and suggest an obvious area for further study.

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13. I thank T. White and M. Fleming for technical assistance. Research supported by the Smithsonian Institution, NSF, NIH, and the University of Missouri–St. Louis.
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Synergy of Ethanol and a Natural Soporific—Gamma Hydroxybutyrate

Abstract. *γ-Hydroxybutyrate and ethanol, as well as γ-butyrolactone and ethanol, are potentiative with respect to duration of loss of the righting reflex (sleep time). The concentration of ethanol in the liver decreases from 30 to 90 minutes after rats are injected with ethanol, but there is no change when ethanol is injected with γ-butyrolactone. In view of the fact that γ-hydroxybutyrate is a natural intermediate in brain, the effects of ethanol on the central nervous system may be mediated through its interaction with γ-butyrolactone.*

Synergistic effects (*1*) on duration of action have been observed between ethanol and cortisone, epinephrine, the phenathiazine derivatives, and the barbiturates (*2*). With cortisone, prior treatment with ethanol causes sleeping time to be merely "prolonged" (*3*); with the other agents mentioned the action seems to be potentiative. γ-Butyrolactone (GBL) has been re-

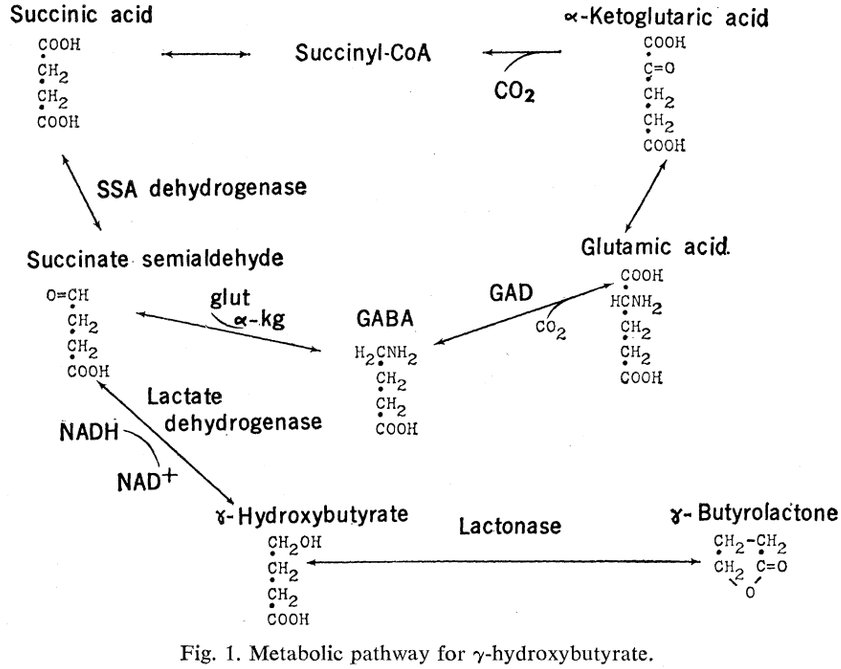

Fig. 1. Metabolic pathway for γ-hydroxybutyrate.

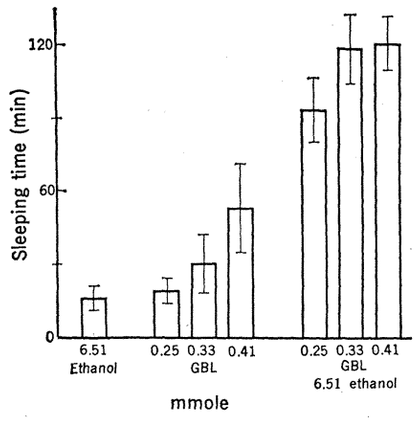

Fig. 2. Sleeping times after intraperitoneal administrations of ethanol, GBL, and the combinations. Sleeping times refer to duration of loss of the righting reflex and the ranges represented by the vertical brackets indicate the standard errors of the means.

ported to be a component of various wines (*4*). It is a soporific agent, identified by Bessman and Fishbein (*5*) and Roth and Giarman (*6*) as an endogenous brain metabolite derived from glutamate through γ-aminobutyrate (Fig. 1). Monnier and Hosli (*7*) have raised the question of whether it is identical with "hypnein," a dialyzable, sleep-inducing humor. Koella (*8*) has discussed this compound as a possible endogenous hypnogen. γ-Hydroxybutyrate (GHB), the free acid form, is considered a hallucinogen (*9*); this central activation, however, is said to be accompanied by a loss of responsiveness and amnesia. γ-Butyrolactone is converted to GHB through a hydroxide-catalyzed hydrolysis; this reaction is reversibly catalyzed by a Ca^{2+}-activated and stabilized lactonase that is present in liver and plasma (*10*). The metabolism of GHB proceeds through succinate semialdehyde in a nicotinamide adenine dinucleotide (NAD)–dependent reaction catalyzed by lactate dehydrogenase; the four isozymes that were tested all showed equal activities (*11*). Prior treatment of mice with GHB has been shown to decrease the dose of ethanol required for anesthesia without increasing the toxicity (*12*). The NAD dependence of GHB metabolism suggested its possible metabolic interaction with ethanol.

The first question in our investigation concerned the effect of GBL and ethanol on duration of loss of the righting reflex (sleep time). Thirty-four male albino rats were divided into the following groups (all substances were administered intraperitoneally): ten animals were injected with 6.51 mmole of ethanol per 100 g of body weight (16 ml/kg of a 23.75 percent solution); four animals with either 0.25, 0.33, or 0.41 mmole of GBL per 100 g of body weight (0.75, 1.0, and 1.25 ml/kg of a 25 percent solution); and four were injected with a combination of the 6.51 mmole of ethanol per 100 g plus 0.25, 0.33, or 0.41 mmole GBL per 100 g of body weight. A potentiative relation was observed (Fig. 2). Analysis of variance showed that the animals injected with the two drugs slept longer than either the GBL group or the ethanol group ($P << .001$ for both of these comparisons). The effect was greater than additive, with the combination dose causing a "duration of sleep" equal to 2.3 to 4 times the sum of the sleep times when GBL or ethanol were given separately.

γ-Hydroxybutyrate was also tested for interaction with ethanol with respect to duration of loss of the righting reflex. The same number of rats was used, and GHB was injected in amounts equimolar to the GBL. An even more marked potentiation was observed (Fig. 3). Analysis of variance showed that the combination group differed quite significantly from the groups given GHB or ethanol alone ($P << .001$ for both comparisons). When the sums of the sleeping times of animals injected with ethanol alone and of animals injected with the three levels of GHB alone were compared to the sleep times of animals injected with pairs of these substances at various dosages, an even greater effect was noted than with GBL. In the animals receiving the combination, duration of sleep was 4 to 5 times the sum of the sleep times when individual doses were given.

When the animals awoke (spontaneously), ethanol and GBL were measured. Ethanol was determined by an enzymatic method (*13*) in which alcohol dehydrogenase oxidized the ethanol to acetaldehyde and the NAD was reduced to NADH; GHB and GBL were determined together (in deproteinized supernatants) as GBL by direct gas chromatography [Barber-Colman 5000; 1.8-m U-shaped column packed with 13 percent diethyleneglycol succinate on Gas Chrom P (Applied Science Labs); 150°C with N_2 as the carrier; detector, flame-ionization type]. The rats were decapitated and exsanguinated immediately upon regaining the righting reflex. The serum was separated, and serum protein was precipitated with 3 volumes of 10 percent metaphosphoric acid. The amounts of both ethanol and GBL

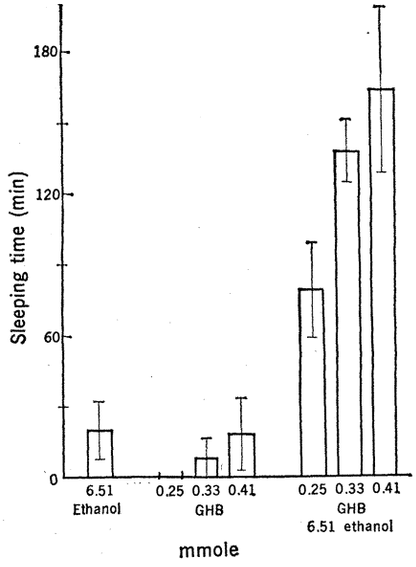

Fig. 3. Sleeping times after intraperitoneal administrations of ethanol, GHB, and the combinations of these. As in Fig. 2, sleeping times are indicated ± S.E.M.

varied from individual to individual in the waking animals. The concentrations of ethanol were approximately the same in the serums of the animals given ethanol or the combination, whereas concentrations of GBL were

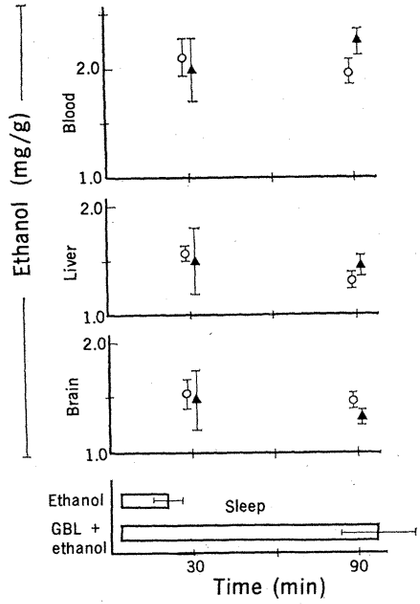

Fig. 4. Concentrations of ethanol in blood, liver, and brain after ethanol or GBL + ethanol. The lower graph shows the data on sleep time, and the upper three graphs indicate the amounts of ethanol in the tissue (○, 6.51 mmole of ethanol per 100 g; ▲, 0.25 mmole of GBL per 100 g + 6.51 mmole of ethanol per 100 g). Values are given ± S.E.M., with eight rats represented in the 30-minute group and four at 90 minutes.

higher in the GBL-injected animals than in the group injected with the combination.

Concentrations of these compounds were next determined at fixed times after an ethanol injection of 6.51 mmole/100 g or a GBL dose of 0.25 mmole/100 g. The animals were killed 30 and 90 minutes later, the approximate waking times of the two groups (Fig. 4). If one compares the effect of ethanol alone with that of the combination for each tissue and each dose, at 30 and 90 minutes, there is only one significant difference. The content of ethanol in the livers of rats injected with ethanol alone is significantly higher at 30 minutes than at 90 minutes ($P < .02$); that is, there is a significant decrease with time. There is no significant decrease in the ethanol concentration in the livers of the combination-injected animals from 30 to 90 minutes, and there is no significant difference between combination- and ethanol-injected animals at 90 minutes. Bustos *et al.* (*14*) have shown that in rats injected with or without pyrazole (a known inhibitor of alcohol dehydrogenase in vitro and in vivo) there is no significant difference in the concentration of ethanol in the blood until 4 to 8 hours after ethanol injections. It might also be added that in the work showing a prolongation of ethanol sleeping time after prior treatment with cortisone there were no differences in concentrations of ethanol in the blood upon waking (*3*).

We have confirmed the observations (*4*) that there is a peak corresponding to GBL in flor sherry (*15*). This peak cochromatographs with standard GBL and is present at approximately 1.5 mmole/liter. This is much less than the amount required for even the lowest level of synergy that we have studied in the rat. For a 70-kg man to ingest GBL to a dose of 2.5 mmole/kg he would have to drink 115 liters of wine.

It would appear therefore that GBL alone does not contribute significantly to the well-known pharmacologic effect of wine. However, we have observed a behavioral interaction of ethanol with GBL and GHB, known soporific components of the central nervous system. The metabolisms of both compounds require NAD, and thus there is the possibility of a competition for this cofactor. Such an antagonism has considerable precedent in the explanations "for many of the known acute effects of ethanol administration" (*16*). This increase in the tissue reductive capacity accompanying alcohol metabolism might tend to favor the conversion of succinate semialdehyde to GHB. There are similarities of actions that might also play roles in the synergy of these compounds. Acetaldehyde and GBL both cause increases in brain acetylcholine (*17*). Hahn and co-workers (*18*) have shown that there is a potentiation of ethanol-induced sleep time with an increase in brain dopamine; and GHB and GBL have been shown to cause increases in brain dopamine (*19*). There is the possibility of an active intermediate or intermediates formed when both substances are present simultaneously.

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Parthenogenesis: Does It Occur Spontaneously in Mice?

Abstract. *If parthenogenesis occurred in bisexual organisms, it would produce an excess of females and depress the sex ratio. The phenotypes of female mice, from matings that produce an excess of females, were examined for evidence of the presence of marker genes of paternal origin. All proved to be hybrids of the maternal and paternal strains, thus excluding parthenogenesis as the cause of the low sex ratio.*

Limited parthenogenetic development of mouse embryos has been observed after electrification of the oviduct (*1*) or treatment of ova in vitro with hyaluronidase (*2*). Edwards (*3*) found some gynogenetic development in eggs of mice inseminated with sperm that had been subjected to various treatments. Gynogenesis is a special form of parthenogenesis that follows activation of the egg by a sperm without contribution of genetic material. Individuals produced by parthenogenesis are called parthenotes (*4*). Successful development from haploid cells would be more likely in the inbred strains of laboratory rodents or their hybrids because recessive lethal genes have been eliminated by inbreeding. The related phenomenon, the development of spontaneous teratomas from male diploid primordial germ cells, is almost unique for mice of inbred strain 129, but teratomas can be induced experimentally in several other inbred strains and hybrids (*5*).

If numbers of parthenotes develop, one would expect to find an altered sex ratio with females predominating. The sex ratio of many inbred strains of mice have been examined (*6*) but only in PHL and BALB/cGnDgWt (*7*) is there a significant excess of females (58.2 and 60.8 percent, respectively). In neither case is the mechanism understood. The abnormal sex ratio appears regularly in the offspring from matings between PHL males and females from other strains but only in certain genotypes of the crosses that use BALB/cWt